Verapamil Improves the Outcome After Cadaver Renal Transplantation


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ABSTRACT

Because of their favorable effects on renal hemodynamics, calcium antagonists may have a major role in the prevention and management of certain types of acute renal dysfunction. In fact, verapamil (VP) was shown to prevent cyclosporin A (CsA)-induced decreases in RBF in mice and in cadaver renal transplant (CRT) recipients. The study presented here of 59 cadaver renal transplant patients evaluates the outcome from perioperative treatment with VP (N = 30) administered intraoperatively into the renal artery (10 mg) followed by oral administration of 120 mg every 8 to 12 h for 14 days versus no drug (N = 29). Early immunosuppression included azathioprine, corticosteroids, and antilymphocyte globulin with subsequent overlapping with CsA on days 5 and 6. Actuarial graft survival at 1 yr was different when the two groups were compared (P < 0.05). Estimated graft survival at 1 yr was 93.3% compared with 72.4% in control patients. The improved graft survival was most striking in repeat transplants with 90% graft survival at 1 yr for VP recipients versus 37.5% for controls. Compared with controls, VP recipients had significantly improved renal parenchymal diastolic blood flow velocities on the first day after surgery (7.8 versus 5.8 cm/s). By day 7, GFR were greater with VP (44 ± 29 mL/min) versus controls (28 ± 22 mL/min). Of VP patients, 67% (18 of 24) had GFR greater than 30 mL/min versus 33% (9 of 26) for control patients. Similarly, on the seventh day, 77% (21 of 30) of VP patients had serum creatinines less than 2.0 mg% versus 34% (10 of 29) for controls. This improved renal function occurred despite significantly higher CsA blood levels in VP recipients, 178 ± 107 versus 89 ± 46 ng/mL in controls. The incidence of delayed function was 10% (3 of 30) and 24% (7 of 29) for VP versus no drug. The improved outcome after cadaver renal transplantation from peroperative VP may be related to its cellular protection from ischemia, the preferential dilation of the afferent arteriole, elevated CsA blood levels, and inherent immunosuppressive properties. It is concluded that VP markedly improves the outcome of cadaver renal transplantation.

Key Words: Calcium channel blocker, RBF, ATN, graft survival, GFR

Aside from their role in treating hypertension, calcium antagonists (CAT), because of their favorable effects on renal hemodynamics, have a role in the management of certain types of acute renal insufficiency (1,2). We have recently demonstrated that CAT such as verapamil (VP) and isradipine prevent cyclosporin A (CsA)-induced RBF inhibition, both in mice (3–5) and in humans (6–8). Furthermore, many CAT, including VP, induce elevated CsA blood levels (8–11). Despite the elevated CsA blood levels, kidney function was improved with CAT as determined by serum creatinines and GFR (8,11). Finally, VP and other CAT might improve immunosuppression because of the inhibition of lymphocyte proliferation in vitro (12–18). Prolonged cardiac allograft survival in rats by VP and CsA supports this notion (19).

The randomized clinical study of cadaver renal transplant (CRT) recipients presented here was undertaken to evaluate early kidney outcome including graft survival after perioperative administration with the calcium antagonist VP.

MATERIAL AND METHODS

Between January 1, 1989, and January 6, 1990, 81 adult CRT were performed. Fifty-nine patients...
with no history of CAT usage were randomized to receive intraoperative VP \((N = 30)\) or no drug \((N = 29)\) (Table 1). Twenty-two patients were excluded from the study for the following reasons: preoperative chronic CAT treatment \((N = 18)\), cardiac arrhythmia \((N = 1)\), no consent \((N = 1)\), and failure to randomize \((N = 2)\). Control patients were allowed other antihypertensive drugs, i.e., angiotensin-converting enzyme inhibitors and clonidine but no CAT.

Methylprednisolone \((375 \text{ mg i.v.})\) was given on the day of surgery \((\text{day } 0)\) and was tapered to prednisolone \((20 \text{ mg orally [p.o.] per day})\) by postoperative day 10 (Figure 1). Azathioprine \((100 \text{ mg p.o.})\) was given on day 0 and was decreased to 25 mg/day for 5 days. Minnesota antilymphocyte globulin (M-ALG) \(15 \text{ mg/kg/day i.v.}\) was given on postoperative days 1 through 5. In the presence of adequate renal function (not requiring hemodialysis), CsA was begun at 7 mg/kg p.o. on day 6 and increased to 12 mg/kg p.o. (day 7). M-ALG was continued during this time to overlap CsA therapy while an effective CsA level was being obtained. Thereafter, CsA doses were adjusted on the basis of kidney function and CsA trough levels (whole blood HPLC). CsA was given once per day at 10:00 a.m. unless CsA blood levels (HPLC whole blood) were greater than 300 ng/mL with increasing serum creatinine.

After restoration of RBF after completion of the vascular anastomoses, VP was given intra-arterially into the renal artery by using a 30-gauge needle and was injected in 2.5-mg increments up to a 10-mg total dose. The intraoperative dose of 10 mg was chosen on the basis of our previous experience that this dose is required to improve turgor and color of the kidney. The 2.5-mg increment administration was used as a precaution against hypertensive episodes. Oral VP was then instituted on postoperative day 1 in a dose of 120 mg (slow release) twice daily for 14 days. The oral dose regimen was given to ensure a constant blood level in a dose that is safe but that approaches maximal for this patient population. By using Doppler ultrasonography (Diasronics Model DRF-400V, 5MHz probe; Diasronics, San Jose, CA), subcapsular cortical parenchymal diastolic blood flow velocities were obtained. The parenchymal flow was measured at 10 mm beneath the kidney surface. The probe reflects the red cell movements expressed in centimeters per second. These measurements were automatically corrected for the probe angle as described before (6,8). Serum creatinine was measured daily during hospitalization, and GFR was obtained on days 1 and 7 with s.c. \[^{125}\text{I}]\text{i}odothalamate \((20)\).

**Statistical Methods**

Standard methods were used to calculate mean and SD in the text and SE of the mean (SEM) in the figures. Comparison of patient demographics and intraoperative data of the two study groups was made by using an unpaired \(t\) test or Fisher's exact test depending on the product-limit method, and comparison of survival curves was made by using the log route test. The unpaired \(t\) test was used for comparison of serum creatinines, GFR, CsA levels, and flow velocities. Fisher's exact test was used to compare graft losses in each group. Results of statistical tests with \(P < 0.05\) are considered significant.

![Figure 1. Outline of immunosuppression protocol and study design. Pred, prednisolone; AZA, azathioprine.](image-url)
RESULTS

Patient demographics and intraoperative data for the two patient groups are reported in Table 1. There were no significant differences between the two groups of subjects with respect to these variables.

GS

Current (May 15, 1991) graft-survival (GS) for VP patients is 87% (26 of 30), significantly better than for that of control patients or 66% (19 of 29) \( P < 0.01 \). Among subjects with current GS, mean follow-up time is 17.1 ± 5.4 months (range, 11 to 28 months) for the control group \( (N = 19) \) and 17.9 ± 5.8 months (range, 11 to 28 months) for the VP group \( (N = 26) \). The difference in GS was most evident for repeat transplants where only 3 of 8 (38%) control patients currently have their grafts versus 9 of 10 (90%) for VP recipients \( P < 0.01 \). These differences were also confirmed in an actuarial GS analysis for all patients \( (P < 0.05) \) (Figure 2A) as well as for repeat transplants only \( (P < 0.026) \) (Figure 2C). GS for first transplants, receiving VP versus control, was not significantly different (Figure 2B). Estimated 1-yr GS is 93.3% among the VP subjects but only 72.4% for the control group. Among repeat transplants, an estimated 37.5% of the control patients retain their graft at 1 yr compared with 90% for VP recipients.

Kidney Function

When patients with GFR of >10 mL/min within 3 days posttransplant were considered, kidney function was significantly improved with VP \( (N = 26) \) treatment. In patients receiving VP, serum creatinine (Scr) fell by 2.7 ± 2.3 mg/dL between postoperative days 1 and 2 or from 7.1 ± 3.2 to 4.4 ± 2.3 mg%, versus 1.8 ± 1.5 mL% for control patients \( (N = 22) \) from 8.6 ± 4.2 to 6.7 ± 3.9 mg% (Figure 3A). On both days, Scr was significantly lower in VP recipients \( P < 0.05 \). By day 7, 77% of the VP patients \( (23 \text{ of } 30) \) had Scr less than 2.0 mg% versus 34% of control patients \( (10 \text{ of } 29) \) \( P < 0.001 \) (Figure 3B). When patients with delayed function (GFR <10 mL/min) were excluded, Scr at day 7 for VP treatment \( (N = 26) \) was 1.5 ± 0.5 versus 2.5 ± 1.7 mg% for control patients \( (N = 22) \) \( P < 0.01 \). Similarly, the improved renal function induced by VP was evident by GFR values \( P < 0.01 \). On postoperative days 1 to 3, GFR for VP patients was 35 ± 25 versus 19 ± 19 mL/min for controls, and on days 7 to 9, GFR in VP patients had increased to 44 ± 22 versus 28 ± 22 mL/min for controls (Figure 3C). Only 6.7% \( (2 \text{ of } 30) \) of VP patients required hemodialysis treatment within the first week versus 25% \( (7 \text{ of } 29) \) of controls.

CsA Levels

Despite similar CsA dosage to all patients, CsA blood levels were almost two times higher in VP patients during VP administration (Table 2, Figure 4A). The mean CsA blood levels during days 7 to 9 after surgery, that is, 3 to 5 days after CsA initiation, were 178 ± 106 ng/mL for VP versus 89 ± 46 ng/mL for controls \( P < 0.01 \) (Figure 4A). CsA blood levels were also significantly different between the two groups from days 7 to 9 through days 16 to 18 after surgery \( P < 0.02 \).
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Figure 3. (A) SCr was lower on days 1 and 2 after transplantation for VP-treated patients compared with control CRT recipients (P < 0.05). (B) By day 7, 77% (23 of 30) of VP had SCr less than 2.0 mg% versus 34% (10 of 29) of control patients ($P < 0.001$). (C) Similarly, GFR was greater on days 1 and 7 in VP-treated patients compared with controls ($P < 0.01$).

RBF

The parenchymal diastolic blood flow velocities on day 1 after surgery were higher in VP patients (8.8 ± 2.9 versus 5.8 ± 3.2 cm/s) than in control patients ($P < 0.001$). Only 2 of 25 (8%) of the VP patients studied had parenchymal flow velocity less than 8 cm/s versus 13 of 24 (54%) for no drug ($P < 0.001$) (Figure 4B). The better flow characteristics were maintained during the VP treatment compared with controls, especially after the introduction of CsA on days 5 and 6.

Urine Flow

Urine volumes, during the first 24 h, were marginally greater in VP patients (6.7 ± 12 mL) than in controls (5.1 ± 8 mL) ($P = 0.06$) (Figure 4C). There were also no differences in urine volumes between the two groups after 24 h for up to 7 days.

Blood Pressure

There was no difference between systolic or diastolic blood pressures between VP and control patients (Table 2). No patient developed signs of hypotension during surgery as a result of VP injection. Occasionally, the systolic blood pressure dropped 10 to 20 mm Hg, followed by rapid return. VP was never discontinued for developing hypotension.

Mortality

Currently (May 15, 1991), there has been no mortality among the 30 VP-treated patients. Three patients in the control group died 6 wk, 4 months, and 12 months after transplantation. Causes of death were intestinal perforation, pneumonia, and liver failure.

DISCUSSION

This study demonstrates several benefits of the perioperative use of VP in CsA-treated CRT recipients. Most importantly, GS and kidney function are improved. Since the completion of the study presented here, these results have been corroborated by a current 96% GS in CRT recipients with VP peroperatively. These beneficial effects may be due to several actions of CAT occurring separately or in combination. CAT improve RBF and prevent acute CsA nephrotoxicity. CAT also have direct and indirect effects that increase the level of immunosuppression.

Calcium plays a key role as a second messenger, regulating lymphocyte activation and proliferation. Calcium influx through membrane channels and mobilization of intracellular calcium stores produce a rapid rise in available cytosolic calcium which helps mediate lymphocyte activation (21). VP and other CAT induce blockade of cellular calcium influx, which inhibits lymphocyte activation and macrophage proliferation in both animal and human in vitro systems (13,15,17,18). These actions may account for the observed decrease of acute rejection episodes and increased GS in VP-treated CRT recipients (8,22). Thus, CAT may directly block immune activation.
| TABLE 2. Daily SCR, systolic (S) and diastolic (D) blood pressures (BP), CsA dose, and hematocrit (Hct) readings in 30 patients receiving VP and 29 control (C) kidney transplant recipients* |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Post-operative day              | Pre-operative   | 1               | 2               | 3               | 4               | 5               | 6               | 7               | 8               | 9               | 10              | 11              | 12              |                 |                 |                 |
| CsA dose (mg/kg)                |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| VP                              |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| C                               |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| ALG (mg/kg)                     |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| VP                              |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| C                               |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| SCR (mg/100 mL)                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| VP                              |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| C                               |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| BP (mm Hg)                      |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| S VP                            |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| C                               |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| D VP                            |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| C                               |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| Hct (%)                         |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| VP                              |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| C                               |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| WBC (no./mm³)                   |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| VP                              |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| C                               |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| Platelets (no./mm³)             |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| VP                              |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| C                               |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| * Values are mean ± SD. WBC, white blood cells.
Figure 4. (A) Blood CsA concentrations in VP patients were almost two times higher compared with controls ($P < 0.01$). (B) Of 25 patients studied, 92% of VP-treated patients had parenchymal diastolic blood flow greater than 8 cm/s versus 43% of 23 control patients ($P < 0.001$). (C) The increased urine output the first 24 h was most pronounced during the first 8 h after surgery in VP-treated patients.

Various CAT have complex and incompletely understood effects of CsA metabolism and CsA blood levels that may indirectly increase the level of immunosuppression. Both diltiazem and VP compete with CsA for the cytochrome P-450 pathway (9,10). Thus, in the presence of CAT, CsA metabolism is decreased and CsA blood levels are increased. At least part of the beneficial effect of these two CAT on transplant outcome may be due to an increased CsA immunosuppressive effect without accompanying nephrotoxicity. It is unclear whether this occurs because of a selective increase in a non-nephrotoxic metabolite of CsA or is because of abrogation of CsA-induced renal ischemia (see below). Although diltiazem has little direct in vitro immunosuppressive activity at concentrations of drug that can be achieved in vivo, it is reported to cause decreases in interleukin-2 and in interleukin-6 levels in CRT recipients (23). Diltiazem also produces a decrease in the M1 and an increase in the M17 and M21 metabolites of CsA (23). CAT may increase various CsA metabolites in the lymphocyte that are immunosuppressive. In contrast to diltiazem and VP, the dihydropyridine CAT nifedipine does not increase CsA blood levels (8–11,24). It remains to be determined if this is a consistent finding for other CAT of the dihydropyridine group. Nevertheless, nifedipine is immunosuppressive in a rat heart transplant model (25).

Calcium is also involved in cellular processes that may be important for renal allograft function and that are separate from immunosuppressive actions. Calcium mediates the biochemical events of smooth muscle tone and cell membrane permeability. CAT are effective antihypertensive agents in the general population and are particularly useful in the many renal transplant recipients who require medical therapy for blood pressure control (26).

CAT improve organ blood flow and offer protection from cellular ischemia. We previously demonstrated, by in vivo fluorescence microscopy in mice, that VP reduces or prevents CsA-induced decreases in red blood cell velocity (3,4). We subsequently confirmed these findings in the clinical setting in CRT recipients (7,8). The study presented here reconfirms the protective effect of VP against CsA-related decreases in RBF. The study further shows that VP offers protection against ischemia and hypothermia that occur during organ procurement, perfusion, and preservation with solutions containing high intracellular range potassium concentrations (1,27). Ischemia may lead to a series of harmful events for subsequent allograft function. First, ischemia produces increases in intracellular calcium that are harmful to the biochemical integrity of the cell (2). This calcium overload produces mitochondrial malfunction and eventually leads to cell death (28). VP increases survival of ischemic renal tubular cells in tissue culture (29). The influence of VP in assisting the rapid restoration of microcirculation after revascularization likely decreases the so-called reperfusion injury from free radicals (30). At pharmacological levels, VP and diltiazem diminish CsA uptake into renal proximal tubule cells in cell culture (31,32). The clinical relevance of this finding at lower doses of CAT is speculative. In all likelihood, each of these effects of VP in
reducing ischemia and entry of CsA and calcium into the hypoxic cell is important and occurs simultaneously (27,33,34).

In several studies, CAT preferentially cause vasodilation of the preglomerular renal vasculature (1,27,34). This site selectively is controversial as diltiazem and VP were recently reported to cause afferent and efferent vasodilation in rats (35). CAT-mediated preglomerular vasodilation renders these agents particularly useful in countering the tendency of norepinephrine-, potassium-, or CsA-induced vasocstriction (1,27,34).

The effect of VP in reducing renal ischemia may decrease allograft immunogenicity. In a murine system, ischemia was found to increase allograft class II antigen expression (36).

In the study presented here, VP use was not associated with any intraoperative hypotension. This may relate to our protocol of aggressive intraoperative blood volume expansion with albumin at a dose of 1.0 to 1.5 g/kg body wt to maintain a central venous pressure of 15 to 18 cm of H2O during surgery (37). Volume expansion allows for rapid increase in blood flow in response to CAT-induced vasodilation.

The study presented here strongly supports routine perioperative use of CAT in CRT to improve renal function and GS. Although VP produces higher CsA blood levels, acute nephrotoxicity is rarely seen and CsA doses are not empirically lowered. The greater degree of CsA immunosuppression without toxicity likely plays a role in the improved results. Some investigators have steadily reduced CsA dose to minimize cost and possibly chronic nephrotoxicity (38). Routine decreases in CsA dose, based on CsA blood levels, may have played a major role in the lack of benefits from CAT reported in other studies (39). The argument could be made not to reduce the CSA dose and to accept higher CsA blood levels without nephrotoxicity and gaining from increased immunosuppression. Better renal function and GS vastly outweigh a small monetary gain from decreased CsA dosing. Finally, CAT should be employed as first-line antihypertensive therapy in CsA-treated CRT recipients.

SUMMARY

VP helps restore and maintain RBF and minimizes renal injury associated with organ procurement and cold ischemia. This randomized clinical study confirms our prior animal findings that VP prevents CsA-associated deterioration of RBF. VP-treated patients have improved RBF and improved renal function despite elevated CsA blood levels. VP given intraoperatively into the renal artery reduces the incidence of posttransplant delayed function. VP-treated patients have fewer rejection episodes, and most importantly, VP is associated with improved GS. The beneficial effect of VP on renal transplant outcome may be related to cellular protection from ischemia, preferential vasodilation of the preglomerular arteriole, elevated blood CsA levels and inherent immunosuppressive properties. It is concluded that VP markedly improves the outcome of CRT.

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